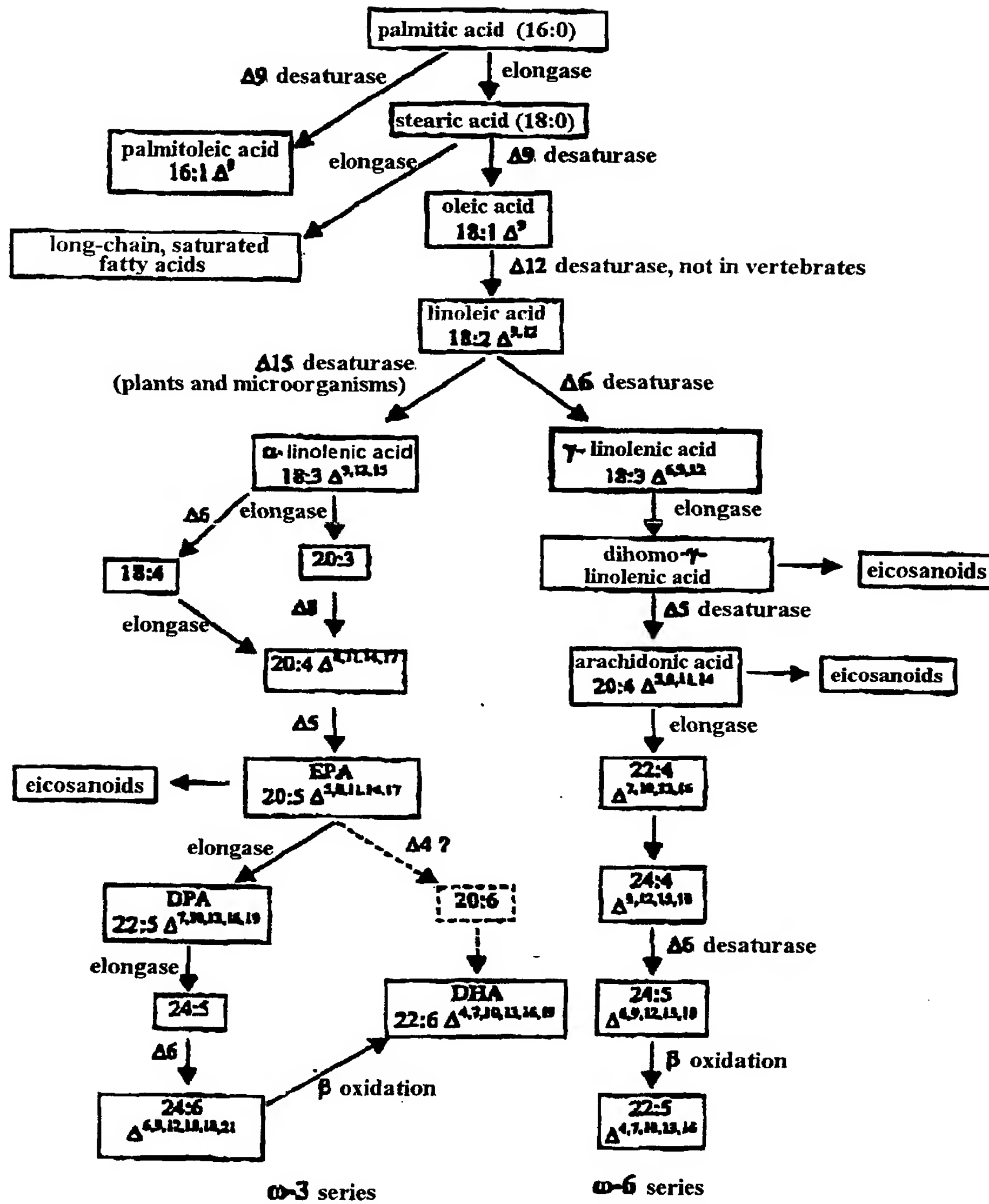


Fig. 1



BLAST 2.0.0 (Jan-05-1999)
References: Altschul, Stephen F., Thomas L. Madden, Alejandro A. Schaffer, Jinghui Zhang, Zheng Zhang, Webb Miller, and David J. Lipman (1997), "Capped BLAST and PSI-BLAST: a new generation of protein database search programs", Nucleic Acids Res. 25:3389-3402.

Query= T. thermophila, delta-6-Desaturase (352 letters)

Database: /LION/data/db/fast/ncdb
387,705 sequences; 119,829,732 total letters

Fig 2

	Score	E	Sequences producing significant alignments:	Value
			(bits)	
15	79	4e-14	trembl AF078796 AF078796_1 gene: "des-3"; product: "delta 5 fat...	
	79	4e-14	trembl AF031477 AF031477_1 product: "delta6-fatty-acid-desatura...	
	78	1e-13	trembl Z81122 CCT13F2_1 gene: "T13F2.1"; Caenorhabditis elegans...	
	78	1e-13	trembl Z70271 CEN08D2_2 gene: "W08D2.4"; Caenorhabditis elegans...	
	70	3e-11	trembl AF005096 AF005096_1 product: "desaturase/cytochrome b3 p...	
	69	6e-11	trembl AJ222980 PFAJ2980_1 gene: "des6"; product: "delta6-acyl-...	
	67	2e-10	trembl U79010 BOU79010_1 product: "delta 6 desaturase"; Borago...	
	67	2e-10	trembl AC005397 AC005397_14 gene: "T3F17.14"; product: "putativ...	
	66	4e-10	trembl AF007561 AF007561_1 product: "delta 6-desaturase"; Bora...	
	64	2e-09	trembl new AF126799 AF126799_1 product: "delta-6 fatty acid desa...	
25	63	3e-09	trembl new AF126798 AF126798_1 product: "delta-6 fatty acid desa...	

2A10

30	tramb1 AF031194 AF031194_1 gene: "S276", product: "S276", trit...	62	6e-09
	tramb1new AB021980 AB021980_1 product: "delta-6 fatty acid desm...	62	6e-09
	tramb1new AL078610 SCS35_12 gene: "SCS35.42c", product: "putati...	62	8e-09
	tramb1 AJ2224160 BNAJ4160_1 gene: "ald1", product: "delta-8 sphi...	60	2e-08
	tramb1 AC004770 AC004770_2 product: "SC269730_2", Homo sapiens...	60	3e-08
	tramb1 AJ224161 MTAJ4161_1 gene: "ald1", product: "delta-8 sphi...	59	6e-08
	tramb1new AL050110 BHH800210_1 gene: "DXT9386C201", product: "...	57	2e-07
35	tramb1 AB022097 AB022097_1 product: "delta 5 fatty acid desatur...	57	2e-07
	tramb1 X07143 HACTY83RN_1 product: "cytochrome b5 containing fa...	50	2e-05
	tramb1 X08460 HSHDE9_1 gene: "Hden", product: "Hden protein", ...	50	3e-05
	tramb1 AF001394 AF001394_1 product: "fatty acid desaturase/cyto...	46	4e-04
	tramb1 AF002668 HSAF2668_1 product: "HLD", Homo sapiens putati...	46	5e-04
40	swiss Q08871 LALCO_HYNY3 LINOLEOYL-CoA DESATURASE [EC 1.14.99.25...	43	0.003

Continuation of Fig. 2

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Fig 3A

[illegible]

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>trembl|AY031477|AY031477.1 product: "delta6-fatty-acid-desaturase",
Caenorhabditis elegans delta6-fatty-acid-desaturase mRNA, complete cds. //:wp|AY031477|3088520
product: "delta6-fatty-acid-desaturase"; Caenorhabditis elegans delta6-fatty-acid-desaturase
mRNA, complete cds. length = 663
```

Score -- 79.2 bits (192), Expect -- 4e-14
 Identifications = 100/390 (25%), Positives = 132/390 (33%), Gaps = 95/390 (24%)

Query: 41 UNREPLYPLMUTIOFORCOSTVYVITL9---RPAALC171497FTEALOS--STZSEKER---93
 ABYCC D Y F H S O A K L K C Z K + KQ
 Subject: 28 KXPCNAVTEU-----RNBDAITHIAPAFBZSSDAIVUOLALNKKOZSDZVSECELEKON 04

[illegible]

Query: 131 R--WFF-SIEPVLVIRISNIGIENR-----WPIUKTAVLAP/CCTHNNWWEK 163
WT S LQ C CW+ H H + P+ +L+ GTE W9 K
Objec: 145 YLEWITRICIALALANQWFWZBNCICDHTFHPYUJISFTCFUCQIRBNWKN 204

Query: 104 INFO:*****[*****]*****CHRYSLER PPL 213
 RM 08 77 Y D DI ON Y P L
 Subject: 205 INFO:*****[*****]*****FEDERAL RESERVE 264

Query: 214 LKWKLSGICADYYE ----- 3 + 48 M
+ I + Q + E + R +
Object: 265 PBNICQSDVPTTCTC-----QSTTKVLRHQALWYATLFLPLTWLPZLS 324

Query: 252 ELIAGTAVLVCHEHDEMF--E8R7LTTEEDIASNOTATHTDIFETALWGGMOYO 309
++ G A ++ MB + K+ NI P QI +NW L GG+ YQ

Query: 326 EELAAATITVCGSNO DQAQSBEJIAANINSGVAKVSFVNLMNIVALLVLCTGTCGTTPFIION-NCGLYON 309

Subject: 384 ICHLITFTHFNCNLCACVATVVAJCKZKZNL 413
 ENR FP + P L + B K+ NL

30

>trambi\079010\00079010 1 product: "delta 6 desaturase", Borago officinalis delta 6 desaturase mRNA, complete cds. 7/9p\079010\00079010 product: "delta 6 desaturase", Borago officinalis delta 6 desaturase mRNA, complete cds. length = 448

Score = 67.1 bits (181), Expect = 2e-10
 Identities = 100/416 (243), Positives = 136/416 (37%), Gaps = 102/416 (24%)

Query: 6 TDSLVJWZBFLMNTYIYEDTSDCTSYALGNNF--CUL7MLUPTDSD08.TEYHT 69
Y #+ +RE + + TO ++ K MVEG L Q++P F
Subject: 10 ZBDLHNNR---COLMBICQCALVTDVED--SPGGF7LMJAC--DTVDJVA 62

Query: 66 LNUKQALVTLVFFKTCANQKQ---FLLH-----KPSLALGGLHLLFVFFPIL 112
 H K L P C G ++ ++ H H + + K K + F I
 Subject: 63 TSPALVTEKQDF-FSTYVLEQVTFVHEDVLELVFFVTEKALVTFKCHINQVATCTA 121

667 ALANRTPD89ALVLEQUSLWSDIATNVCSELT--BNAACBOYALANVTOR 221 :32(q
++ A1 7 + H B0LOS I + *1 G4 7 O J1 + ++ +
091 LATVNVTIEE--VMSMKNULGCSITIBVA3T4ICUAKBSCTOOLAVLE-ALS7STJ01 C11 :Kzomr

[illegible][illegible][illegible]

Subject: 206 INASHTATVOTFOLJINGDNDIVTMSIT7012PTULPQWVIZAKKXCNL 339
 ++ FI CS+OQ OM PT+P L E + E XZ WL
 Subject: 339 CPMON-TH-----CMTGVNBSJ77WNSHCHSLOVIVGVIKXNL 403

**DE
FILE**

40
 45
 50
 55
 60
 65
 70

36

>cds|090971|L2C0 8703 LIPOLYTIC-CD3 DESATURASE (EC 1.14.99.25)
(NC037456)-desaturase). //tr|tr|L11471|806003 1 product: "delta-6 desaturase"; Synchocystis
sp. delta-6 desaturase gene, complete cds. //tr|tr|L09310|83914 112 gene: "des6"; product:
"delta-6 desaturase"; Synchocystis sp. PCC6803 complete genome, 16/27, 1993550-2137258.
//pt|tr|L13157|83157 2 delta-6 desaturase - Synchocystis sp. //cp|090510|163389 gene:
"des6"; product: "delta-6 desaturase"; Synchocystis sp. PCC6803 complete genome, 16/27,
1993550-2137258. //cp|L11021|319563 product: "delta-6 desaturase"; Synchocystis sp. delta-6
desaturase gene, complete cds. Length = 359

Score = 43.4 bits (100), Expect = 0.003
 Identification = 63/200 (21%), Positives = 101/200 (50%), Gaps = 61/200 (21%)

[illegible]

Query: 176 ~~XXXXXXXXXX~~-----XXXXXXXXXX
 1+ 0 +00 02 +20 + 0 +2 0 + 0 . F W L +
 Subject: 115 ~~XXXXXXXXXX~~-----XXXXXXXXXX
 174

Query: 225 YN82031AL-----WVLLTQNTIVILS-----ALACITFASILVZ 285
 .Y+ ++L L E + F 4L L OF 4A0
 Object: 175 YN82031ALACITFASILVZ-----WVLLTQNTIVILS-----ALACITFASILVZ 230

Query: 266 ~~XXXXXXXXXX-71777777~~-----Q1AAK0VAFED1992 100
 $M + 34 \quad R \quad Q1 \rightarrow M + 2 \rightarrow R$
 Objct: 215 ~~XXXXXXXXXX-71777777~~-----Q1AAK0VAFED1992 296

Query: 301 L I N C O M P O S I T E P R O C E S S I N G 348
 C O D + O M I T I P + I I + + + + P P +
 Object: 293 M I C R O S O F T W O R K S H E E T P R O C E S S I N G 312

10

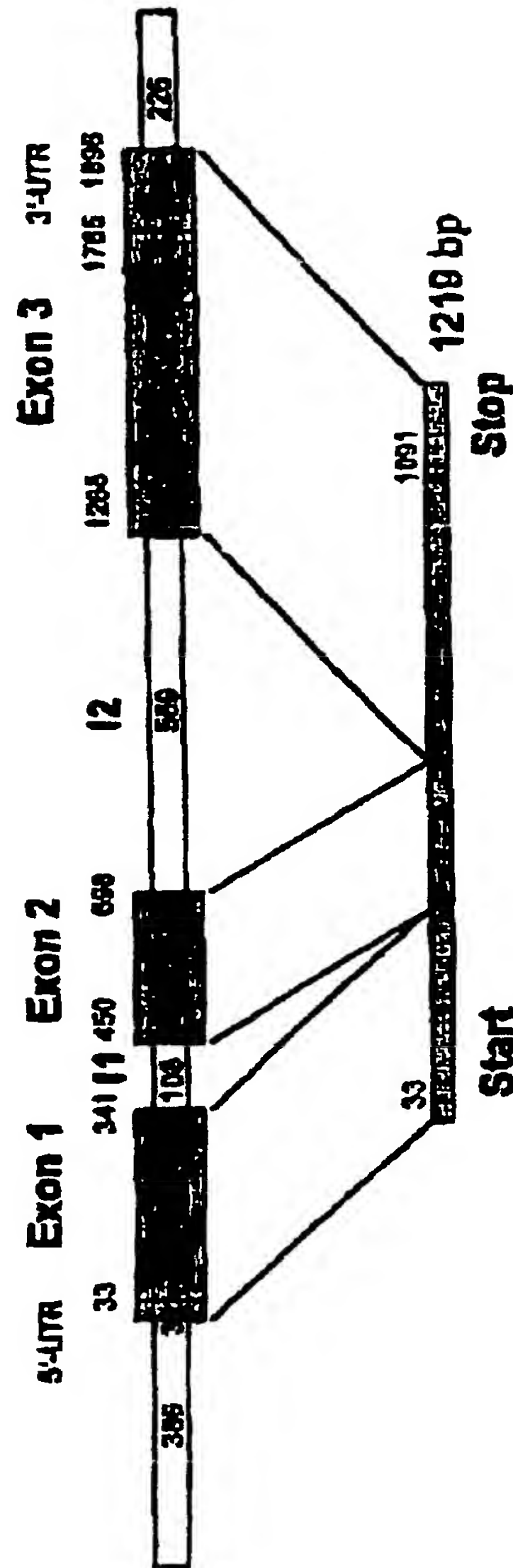
6A/10

[illegible]

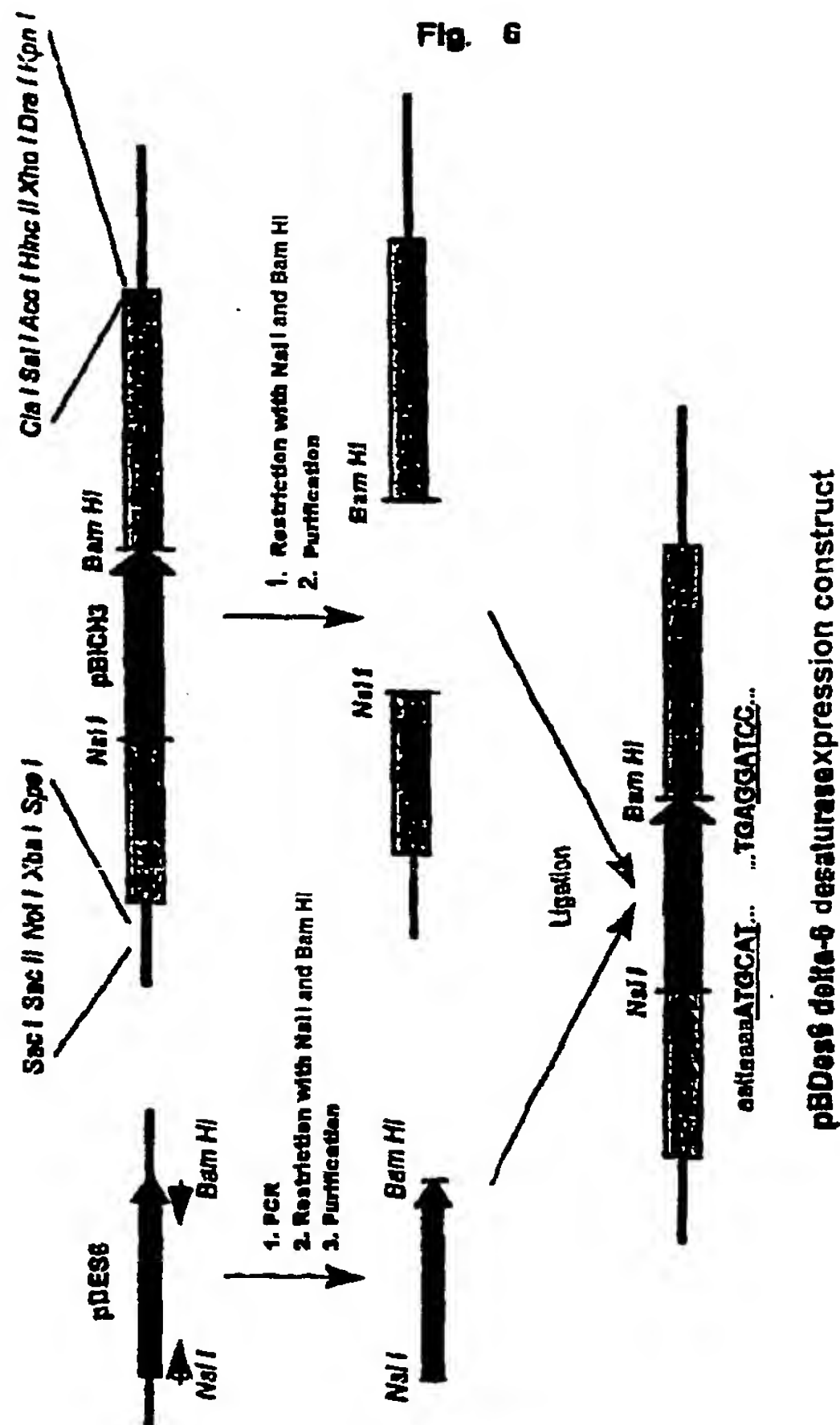
Continuation of Fig. 4

Fig. 5

Structure of the Δ -6-desaturase gene



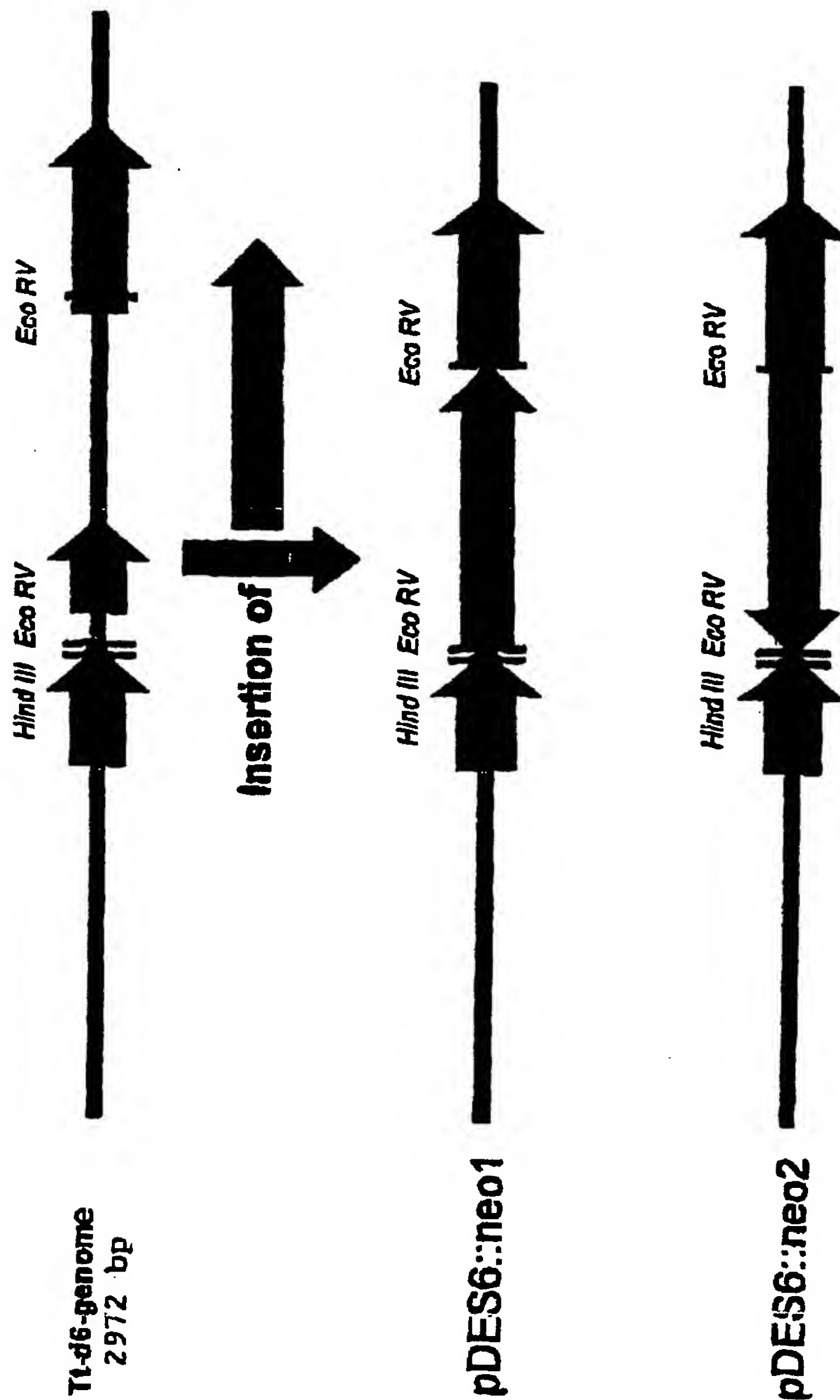
Preparation of the pBDES6 expression construct



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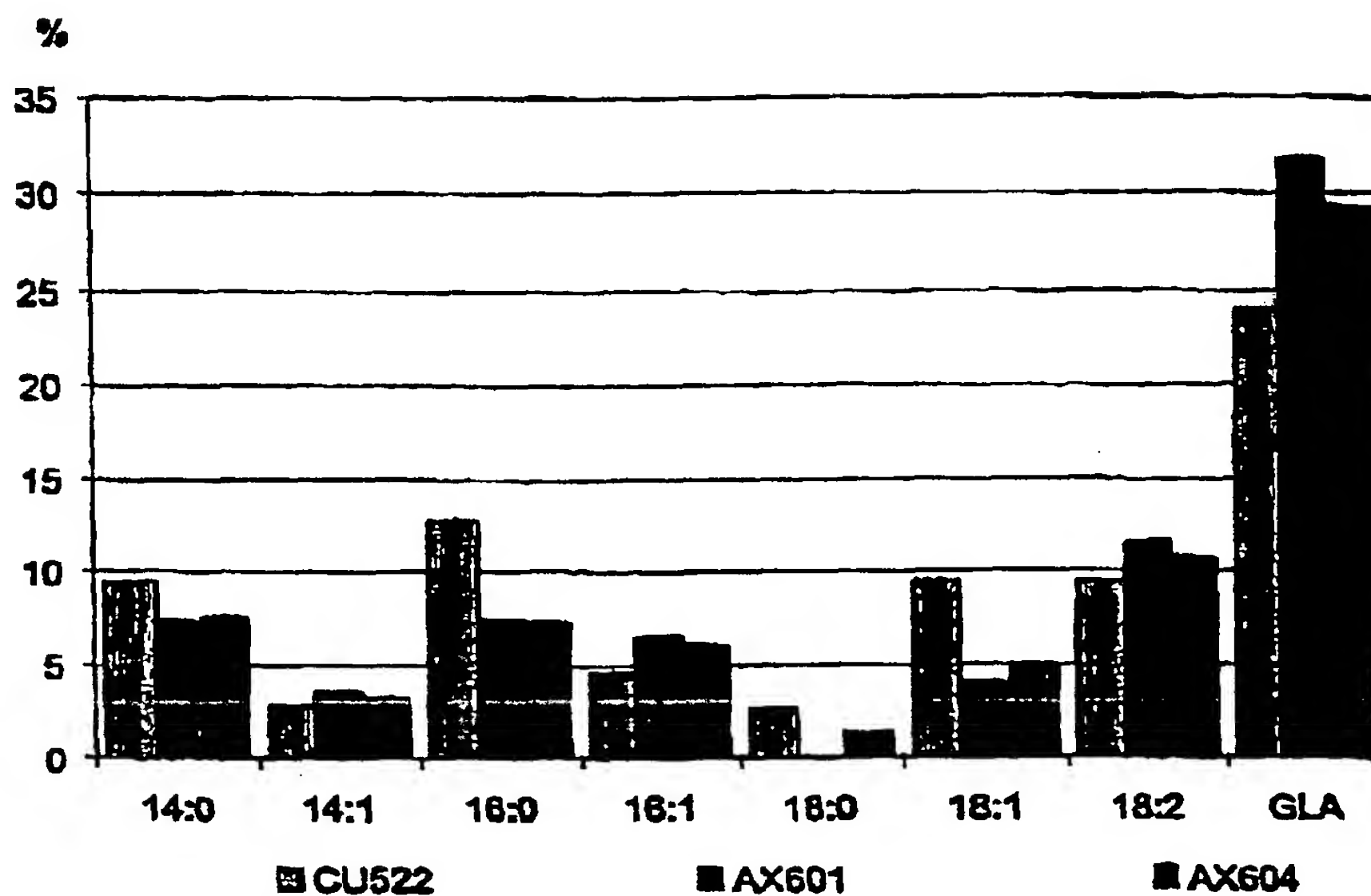
Fig. 7

Delta-6-desaturase knockout construct



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Fig. 8



Comparison of the fatty acid spectrum (main fatty acids) of the Tetrahymena pBDES6 transformants (AX601 and AX604) with that of the Tetrahymena wild-type strain (CU522) after 50 h of culture. The figure gives the percentage content of the main fatty acids in the total fatty acid spectrum